

Comparison of Cells Isolated from Fat Collected by Power Assisted Liposuction (PAL) or Suction Assisted Liposuction (SAL)

September 13, 2011



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CONTRACT SERVICES REPORT

INCELL Project Number(s): MICA_101013

Report Date: September 13, 2011

Project Title: Comparison of Cells Isolated from Fat Collected by Power Assisted Liposuction (PAL) or Suction Assisted Liposuction (SAL)

CLIENT GENERAL INFORMATION	
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SUBMITTED FOR INCELL BY:



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INCELL Chief Science Officer

Date: 09/13/11

As per the Client's specifications this was not done as a GLP study according to CFR21 Part 58, but was for discovery or R&D Use Only. The test results are based on the submitted sample(s) only. Any tests done for "R&D Use Only" are not to be used for regulatory or marketing purposes without prior knowledge of INCELL. The test report shall not be reproduced except in full, without written INCELL approval. It is the Client's responsibility to ensure that it is in compliance with all U.S. federal, state, and local laws & regulations. The liability of the study implementers, INCELL, RENEW and ECT, shall not exceed the fees paid for the testing reflected on this report. All results were kept confidential and will be shared only with the Client's permission.

Comparison of Cells Isolated from Fat Collected by Power Assisted Liposuction (PAL) or Suction Assisted Liposuction (SAL)

I. OVERALL GOAL OF THE STUDY

The overall purpose of this study was to compare the viability, quality and quantity of Adipose stem/stromal fraction cells (ADSCs) derived from fat tissue obtained using PAL or SAL methods on the same patient. Additional goals were to compare surgeon satisfaction in using the two methods and to compile medical and scientific information in a form that can potentially be developed for publication in a reputable medical or scientific journal. This work was done in conjunction with the surgeons at Renew Associates, PA (Dr. Jaime R. Garza, President).

II. PERFORMANCE SITES AND GENERAL APPROACH

There were two performance sites: (A) Clinical; and (B) Bioprocessing and Laboratory. Locations of those sites are noted below. Microaire provided the following Materials and Supplies to the Clinical Site as part of the work: PAL 650 System (handpiece x 2, aspirator, tubing); PAL cannulae-Mercedes style, reusable, 30 total (10 ea. 4mm; 10 ea. 3mm; 10 ea. 2.4mm).

Performance Sites and Approach

A. Clinical (liposuction surgeries; patient and data management; marketing): Liposuction Surgery

Renew Associates PA ("Renew")
21 Spurs Lane, Suite 110, San Antonio, TX 78240

Monitoring and review of Renew site clinical studies and procedures:

Endeavor Clinical Trials (ECT)
Physicians Plaza II, 8042 Wurzbach #420, San Antonio, Texas 78229

Approach. Liposuction was performed at the clinical site with collection of adipose tissue using either the PAL or SAL Methods. Notes were made of the times for collection. A Questionnaire and summary of information was developed to query the surgeons (N=3) who performed the liposuction procedures. Descriptions of the questions developed, the surgeons' answers, and associated outcomes are described in the results. Tissues were labeled and transferred to the laboratory site for bioprocessing.

B. Bioprocessing & Laboratory (overall project management, process tissues, perform laboratory assays, reporting):

INCELL Corporation LLC ("INCELL")
12734 Cimarron Path, San Antonio, TX 78249

Approach. Tissues were rinsed aseptically several times then bioprocessing was done to release the Adipose-derived analyses of cell viability and characterization as detailed in the Study Design and Methods.

III. STUDY DESIGN AND METHODS

A. Clinical Parameters: Patients

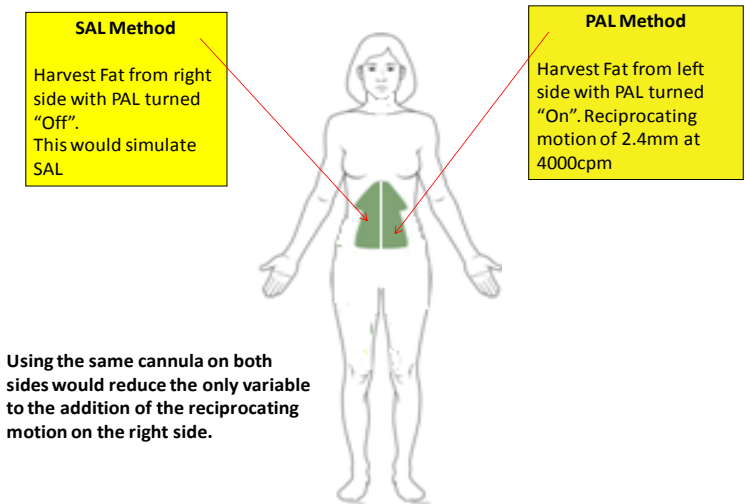
1. Number (N). Patients were recruited and included according to the original study design. Number of Subjects Planned for the Study: N=10. However, 11 patients were actually recruited and received liposuction surgery as described in the results section.
2. Source of Patients. Elective cosmetic surgery patients of the Renew Associates, PA plastic surgery group
3. Consent. All patients who were the tissue donors had a signed consent form agreeing to the research prior to study initiation
4. Patient history. Obtained patient history and general demo-graphics to include sex, age, weight, height, ethnicity, pre-existing or known genetic conditions
5. Inclusion Criteria
 - a. Male or female
 - b. Age 20-50
 - c. Good health; ASA Class I
 - d. BMI < 30
 - e. Eligible for in-office procedures
 - f. No history of bleeding disorders, diabetes, HIV or lipoatrophy disorders (e.g., lupus, scleroderma)
6. Exclusion Criteria
 - a. Age restrictions
 - b. ASA Class II or above
 - c. BMI >30

B. Project Tasks and Subtasks

1. TASK 1: Generally, the Project Management and Reporting adhered to the original sub-tasks, but “e” was modified to increase payments for patient surgeries:
 - a. Refined SOW and subcontracting arrangements
 - b. Signed-off on all paperwork by Principal Investigator and Co-Investigators
 - c. Coordinated IRB relationship and contracts
 - d. Wrote IRB protocols; had meetings, teleconferences and other communications with IRB committee, project participants, and Sponsor
 - e. Revised payment components to pay patient surgeries to accelerate accrual
 - f. Developed all associated project paperwork
 - g. Defined record-keeping parameters and archiving procedures
 - h. Defined data collection and tabulated outcomes; archiving procedures
 - i. Developed consent, transfer and other forms prior to initiating study
2. TASK 2: Marketing Study to Community
Sub-tasks for Task 2 were completed as follows:
 - a. Designed marketing materials
 - b. Conveyed ads to newspapers or magazines and paid for publication
 - c. Gave presentations
3. TASK 3: Clinical Methods
Sub-tasks:

- a. Summarized equipment and materials lists conveyed to MicroAire
- b. Pre-consented eligible patients
- c. Pre-planning: surgery dates were set and laboratory contacted for preparatory work, chain-of-custody & materials transfer
- d. In-office, tumescent, liposuction procedures were done
- e. Payments, including patient incentives, were managed
- f. Oral sedation and local anesthesia were given
- g. Preparation was done for tissue collection and questionnaires
- h. Two comparable sites of each patient, as shown in Figure 1, had fat harvested from left and right sides: One by SAL, the other by PAL Method; The same fat tissue location and approximately the same volume was taken from each Study Subject donor

Figure 1. Diagram of Fat Collection Sites and Methods



- i. Mercedes style cannulas were used: 1 set per patient
- j. Fat tissue samples were collected into separately pre-labeled SAL and PAL sterile containers; Note: the total collection volume and other information was put on collection sheets
- k. Fat was transferred to lab transport carrier
- l. Contacted courier for pickup
- m. Maintained patient confidentiality and HIPAA compliance
- n. Data were analyzed and prepared for potential publication and as report summarizing comparisons between test groups (PAL vs. SAL) from clinical use perspective: Note: using the same cannula on both sides reduced the only variable to the addition of the reciprocating motion on the right side.

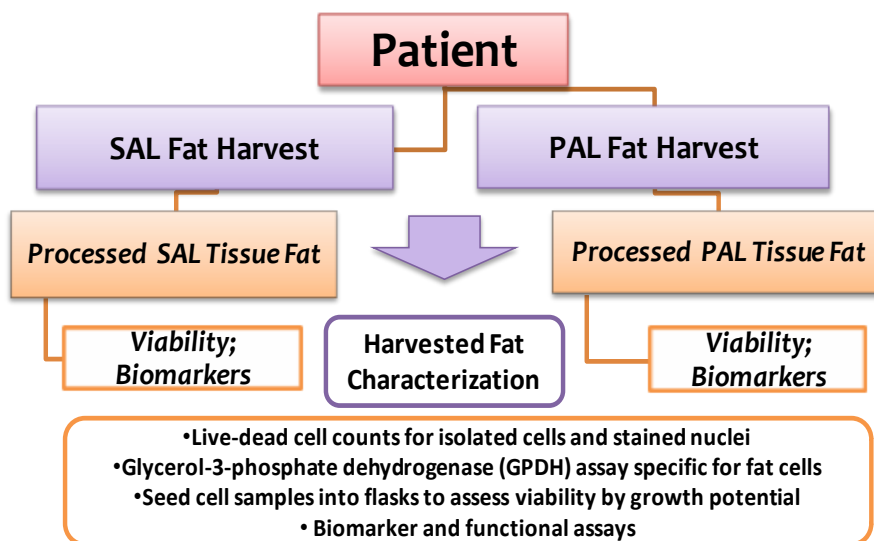
4. TASK 4: Monitoring

Sub-tasks:

- a. Pre-review of consent form documents was done to assure compliance
- b. Pre-review of patient information was done to verify eligibility prior to study inclusion
- c. Coordinated training of surgeons on project compliance and expectations
- d. Reviewed documentation to verify completeness and organization to include use for potential publication and as report summarizing comparisons between test groups (PAL vs. SAL) from clinical use perspective
- e. Verified clinical protocol compliance for each patient by surgeons
- f. Verified compliance with HIPAA and patient confidentiality

- g. Verified complete documentation by surgeons
- h. Validated transfer of materials from Renew to INCELL as per SOP

Figure 2. Flow Diagram of Clinical Methods and Lab Testing



5. TASK 5: Laboratory Testing (See Figure 2 Flow Diagram)

Sub-tasks:

- a. Quality standards were maintained throughout the tissue receiving, processing, cell release from the tissues and laboratory analyses.
 - (i) Reagents and kits were purchased.
 - (ii) Processing reagents were prepared as sterile solutions.
 - (iii) Labeling of components was done.
 - (iv) Communications and chain-of-custody planning was put into place.
- b. Lab transport courier and delivery procedures were defined.
- c. Harvested PAL and SAL tissue pre-digestion by enzymes: test viability
- d. Bioprocessing and enzyme treatment to isolate ADSCs from SAL or PAL harvested tissue: test viability post-digestion
- e. Viability assay methods and cell counts:
 - (i) All cells: Live-dead cell counts (trypan blue dye exclusion) for tissue-associated or isolated cells
 - (ii) Enzymes: Glycerol-3-phosphate dehydrogenase (GPDH) assay specific for viable fat cells; ATP presence of live cells
 - (iii) Seeded cell samples (ca. 1×10^6 cells each into 25-cm² culture flasks to briefly assess viability by growth potential over 2-3 week period
- f. Biomarker Assays:
 - (i) Aldefluor (Stem Cell Technologies Inc.) assays were done to detect aldehyde dehydrogenase activity (ALDH) which is present in all stem and progenitor cells (an enzymatic, non immune assay procedure)
 - (ii) Biomarker immunoassays include a set of markers expected to be positive on ADSCs, including CD105, CD44, CD90 and Stro-1, as well as CD45 (which was expected to be negative). Immunoassays were

optimized for testing cells immediately after isolation and/or on cells cultured in vitro on multi-well Lab-Tek slides.

- g. Data analysis and presentation:
 - (i) Summarized data as graphs and/or tables, with statistics, as appropriate
 - (ii) Photos were selectively taken to represent the observation
- h. Data were developed for this report summarizing comparisons between test groups (PAL vs. SAL)

6. TASK 6: Final Report

This report represents information compiled from Renew, INCELL and ECT. It is a summary and integration of the outcomes data and analysis, including statistical assays and graphics. Color-coded graphics representing specific groups (e.g., SAL=Blue, PAL=red; Individual surgeons are coded green, lt. blue and mauve).

This integrated data and information capture is planned for research and/or clinical publication(s). Also, Microaire may elect to include some of this information in its marketing.

Table 2. Biomarker Antibodies Used in Assays¹

(+) Marker	Antibody	Description
CD44 on ADSCs and other MSCs	Mouse MAb [F10-44-2] Abcam Cat #Ab6124	CD44 is a receptor for hyaluronic acid and can also interact with other ligands (e.g., osteopontin, collagens, matrix metalloproteinases, MMPs). CD44 posttranslational modifications control its function.
CD90 (Thy-1)	Mouse MAb [AF-9] to CD90/Thy1, Abcam Cat #Ab2894	A 25–35 kD GPI-anchored protein of the Ig superfamily. CD90 expression is found on mesenchymal stromal cells (MSCs) ² . CD90+CD34+ cells characterize a highly enriched population of high proliferative potential colony-forming cells (HPP-CFC) ³ .
CD105 (endoglin) ⁴	Mouse MAb [105C02] to CD105, Abcam Cat #Ab44967	A homodimeric integral membrane glycoprotein composed of disulfide-linked subunits of 90-95 kDa; a component of the transforming growth factor- β receptor complex; a biomarker of angiogenesis.
Stro-1 antigen	Mouse Anti-STRO-1 Monoclonal Antibody, Millipore Cat #MAB4315	Cell-surface glycoprotein on subsets of bone marrow stromal (mesenchymal) cells; selection of Stro-1+ cells assists in isolating mesenchymal precursor cells, which are multipotent cells that give rise to adipocytes, osteocytes, smooth myocytes, fibroblasts, chondrocytes, and blood cells.
(-) Marker	Antibody	Description
CD45; LCA also known as Ly-5 or T200	Rabbit polyclonal to CD45, Abcam Cat #10559	CD45 leukocyte common antigen (LCA) is found on all cells of hematopoietic origin, except erythrocytes. CD45 is a transmembrane glycoprotein which is expressed at high levels on the cell surface, and its presence distinguishes leukocytes from non-hematopoietic cells.

¹ <http://stemcells.nih.gov/info/scireport/appendix.asp>

² Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8: 315–317.

³ Mayani H, Lansdorp PM. (1994) Thy-1 expression is linked to functional properties of primitive hematopoietic progenitor cells from human umbilical cord blood. Blood 83: 2410–2417.

⁴ Barbara NP, Wrana JL, Letarte M: Endoglin is an accessory protein that interacts with the signaling receptor complex of multiple members of the transforming growth factor-beta superfamily. J Biol Chem 1999, 274:584-594.

IV. RESULTS

A. Clinical Parameters: Study Subjects

1. Selection, Consent, Accrual and Demographics

Patients were recruited according to the original study design. However, the accrual was extremely slow since patients expected to have the surgery paid for as part of their participation. The contract was therefore renegotiated and the IRB Protocol revised so that the surgery was paid and the surgeons agree to a lower their fees so that the increased study cost was minimized and the study could proceed. Demographics of the Study Subjects are detailed in Table 1.

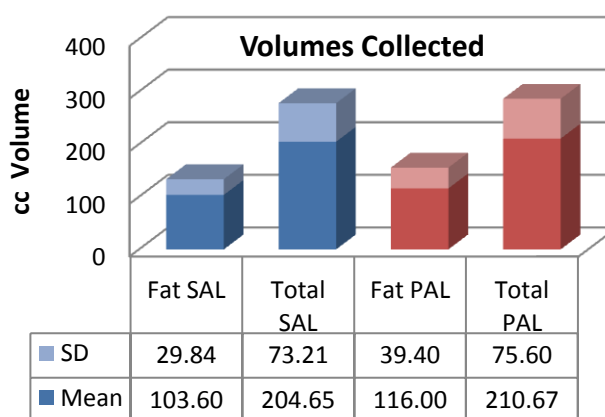
Table 1. Demographics of Study Subjects

Patient	M/F	Age	Ethnicity	Weight (lb)	Height (in)	BMI
#1	Female	30	White/European	153	68"	23.2
#2	Male	44	White/European	160	68"	24.3
#3	Female	25	Latino	130	63"	23.0
#4	Female	40	Latino	123	61"	23.2
#5	Female	51	White/European	150	65"	25.0
#6	Female	45	Latino	140	62"	25.6
#7	Female	24	Latino	170	61"	32.1
#8	Female	35	Latino	189	67"	29.6
#9	Female	24	White/European	128	64"	22.0
#10	Male	24	White/European	195	67"	30.6

2. Number of Subjects

The number planned was 10. However, 11 patients were actually recruited and received liposuction surgery. One patient had sinewy tissue material that was not anything like any fat samples obtained from other patients in this or other studies. Minimal tissue processing was done, but it was agreed that the tissue should be discarded and, thus, no additional laboratory or data collection was done. Also, the data from that patient's surgery was excluded from the summary of surgical procedures, and the 11th recruited patient was substituted in presentation of the data.

Figure 2. SAL and PAL Lipoaspirate Volumes



Legend: "Fat SAL" or Fat PAL refer to the "dry" lipoaspirate remaining after centrifugation and rinsing to remove tumescent fluid. "Total" refers to the total SAL or PAL lipoaspirate volumes collected (fat + tumescent fluid) for N=10 Study Subjects. Values plotted for cc Volumes show Mean and SD.

3. Lipoaspirate Volumes

Figure 2 shows that there were no statistically significant differences (Mean \pm SD) in the fat tissue or the total lipoaspirate volume when SAL and PAL were compared among the tissues harvested from the 10 Study Subjects. SAL Fat volume was 103.6 ± 29.8 whereas PAL Fat Volume was 116 ± 39.4 . Total lipoaspirate for SAL and PAL respectively were 204.65 and 210.67 for all ten test subjects. Table 2 shows details of the liposurgery volumes data from the 3 separate surgeons. It is apparent that there are variable mean values and fat to lipoaspirate total volumes (Table 2).

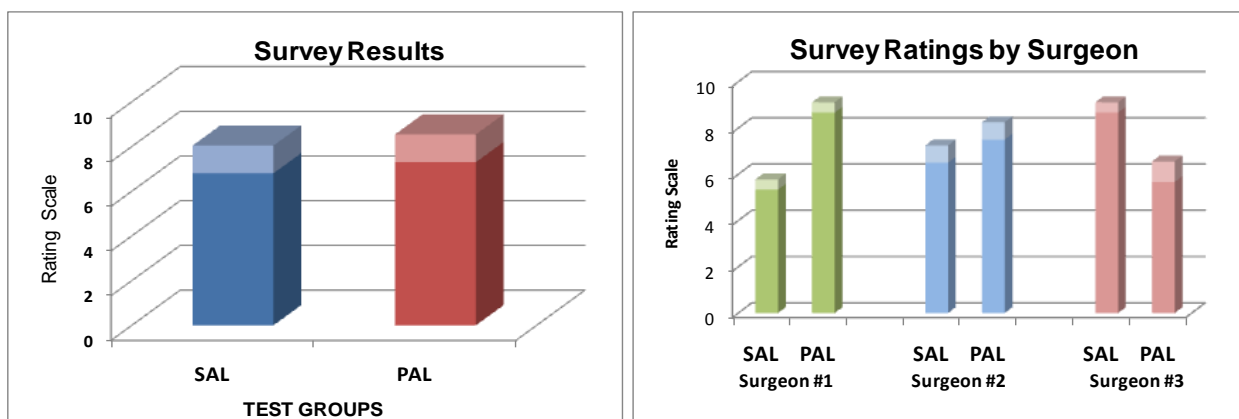
B. Surgeon Surveys

After each surgery, surgeon satisfaction surveys were done to compare SAL vs. PAL using the rating system described in the legend to Figure 3. Outcomes were essentially the same for both groups, and differences were not statistically significant when all data were pooled (Figure 3, top graph).

Table 2. SAL and PAL Lipoaspirates (Total and Fat) Data by Surgeon

Surgeon #1				
Patient	Right SAL	Total Aspirate	Left PAL	Total Aspirate
#2	200	300	275	400
#6	150	170	150	170
#8	110	310	100	300
	Fat SAL	Total SAL	Fat PAL	Total PAL
Mean	153.33	260.00	175.00	290.00
SD	31.11	60.00	66.67	80.00
Mean Vol ratio	SAL/Total Aspirate	0.59	PAL/Total Aspirate	0.60
Surgeon #2				
#1	81	121.5	105	161.7
#3	80	130	80	130
#4	75	150	75	100
#7	100	370	120	310
	Fat SAL	Total SAL	Fat PAL	Total PAL
Mean	84.00	192.88	95.00	175.43
SD	8.00	88.56	17.50	67.29
Mean Vol ratio	SAL/Total Aspirate	0.44	PAL/Total Aspirate	0.54
Surgeon #3				
#5	75	150	75	175
#9	90	170	90	170
#10	75	175	90	190
	Fat SAL	Total SAL	Fat PAL	Total PAL
Mean	80.00	165.00	85.00	178.33
SD	6.67	10.00	6.67	7.78
Mean Vol ratio	SAL/Total Aspirate	0.48	PAL/Total Aspirate	0.48

Figure 3. Surgeon Survey Results



Legend: For each of the 10 patients, the three surgeons answered queries described below for the PAL and SAL use on each side of the patient. The Mean \pm SD values for all observations are shown in the graphs. **PAL:** On a scale of 0 to 10 with 0 being poor, 5 being acceptable and 10 being outstanding, rate the PAL procedure with a number for this patient and harvest. **SAL:** On a scale of 0 to 10 with 0 being poor, 5 being acceptable and 10 being outstanding, rate the SAL procedure with a number for this patient and harvest. Differences in PAL vs. SAL were statistically significant ($p < 0.05$) for Surgeons 1 and 3.

However, there were statistically significant ($p<0.05$) differences in the ratings by the individual surgeons (Figure 3, bottom graph) broken out by each patient. Two of 3 surgeons rated PAL above SAL, whereas the other surgeon rated PAL below SAL, suggesting some personal preference and use differences among the surgeons.

The detailed ratings for each patient and denoted by surgeon # are shown in Table 3. The third question on control of the cannula tip and contouring directly correlated with the relative SAL or PAL satisfaction of the individual surgeons. Nevertheless the overall rating had a mean \pm SD value of 2.9 ± 0.72 which indicates that the procedures are equivalent by ratings.

Table 3. Details of Individual Surgeon Responses to Surveys

Patient Numbers	1	2	3	4	5	6	7	8	9	10		
	Dr #2	Dr #1	Dr #2	Dr #2	Dr #3	Dr #1	Dr #2	Dr #1	Dr #3	Dr #3	Mean	SD
On a scale of 0 to 10 with 0 being poor, 5 being acceptable and 10 being outstanding, rate the SAL procedure with a number for this patient and harvest.	5	6	7	7	8	5	7	5	9	9	6.8	1.24
On a scale of 0 to 10 with 0 being poor, 5 being acceptable and 10 being outstanding, rate the PAL procedure with a number for this patient and harvest.	6	8	8	8	7	9	8	9	5	5	7.3	1.24
Comparing control of cannula tip (i.e., ability to contour) choose one of the following:												
(1) PAL was much better than SAL	3	3	2	2	4	3	2	2	4	4	2.9	0.72
(2) PAL was better than SAL												
(3) PAL was about the same as SAL												
(4) PAL was worse than SAL												
(5) PAL was much worse than SAL												
Legend: Color-coding: Green (Dr #1); Lt. Blue (Dr #2); Mauve (Dr #3); Gray (Statistical analyses of the data sets).												

C. Clinical Monitoring

No major concerns were found during the monitoring of the studies by the Endeavor Clinical Trials (ECT) Team and INCELL QA Coordinator, Lynn Miller. Overall, the consent form documents, patient information, compliance training, and review of study documents were consistent with expectations.

During the course of the study there were some personnel and procedural changes that were adequately addressed through the revised IRB, surgeon compliance, staff compliance, and patient monitoring procedures. There was verified compliance with HIPAA regulations and chain of custody and courier procedures for transfer of tissues to INCELL for processing.

In general the project was well-executed and the laboratory study results were developed from quality clinical source materials.

D. Laboratory Studies

PAL and SAL were processed separately and evaluated in comparative studies by assays detailed in the methods section and in the Figure and Table Legends.

1. Fat Tissue Viability

Harvested PAL and SAL adipose tissues were assessed in viability tests (Table 3) and 100% viability was demonstrated.

Table 3. Viability Testing of Fat and Isolated Cells			
Observations and Assays for Viability Testing	Liposuction Method		
	SAL	PAL	ALL
Fat Tissue Viability	Pos. #/Test # (%)	Pos. #/Test # (%)	Pos. #/Test # (%)
Explant Culture (1)	10/10 (100%)	10/10 (100%)	20/20 (100%)
GPDH Activity (2)	9/9 (100%)	9/9 (100%)	18/18 (100%)
ATP Luminescence (3)	3/3 (100%)	3/3 (100%)	6/6 (100%)
Isolated Cells Viability	Pos. #/Test # (%)	Pos. #/Test # (%)	Pos. #/Test # (%)
Dye Exclusion Trypan Blue(4)	10/10 (100%)	10/10 (100%)	20/20 (100%)
Culture, Expand, Cryo (1)	10/10 (100%)	10/10 (100%)	20/20 (100%)

Legend: (1) All explants and cell cultures were seeded into M3:10™ medium and grown as monolayers in 25-cm² culture flasks, expanded at least once then cryostored (INCELL EZ-CPZ™ medium) in liquid nitrogen (vapor phase); Most were also reanimated from cryostorage and showed high (>80%) viability. ATP: Adenosine triphosphate; GPDH: glycerol-3-phosphate dehydrogenase; (2) all tested except patient #2; (3) only patients 9, 10, 11 were tested (560 nm luminosity program on Spectramax M2 platereader); (4) Observed averages of >80% viability (Trypan Blue assay) after enzyme release and dissociation from tissue.

2. Explant Cultures

Fat tissue viability was demonstrated by explant cultures of cells from the adipose tissues. Tissues explants (ca. 0.5 cm²) seeded into M3:10™ complete growth medium (INCELL) supported outward migration of cells onto the plastic monolayer culture flask from all of the tissue samples. SAL and PAL appearing essentially equivalent after 1 to 3 weeks of growth. Cultures were subcultured no more than 1 to 2 times and were then cryopreserved in EZ-CPZ™ cryopreservation medium (INCELL) in cryovials stored in the vapor phase of liquid nitrogen.

3. Glycerol-3-Phosphate Dehydrogenase (GPDH) Assays

GPDH is an enzyme that is specific for mature fat cells and adipose tissue. It was tested in tissue samples diluted from 1:1 to 1:128 to determine peak maximum activity of the enzyme and potential interference with enzyme activity at lower fat dilutions. Figure 4 exemplifies individual fat sample assays of SAL and PAL from the majority of Subjects in the Study. The Subject numbers are indicated for the individual graphs and there is a composite

graph for all of the data from the test Subjects. All tested samples had activity that peaked at the 1:64 dilution.

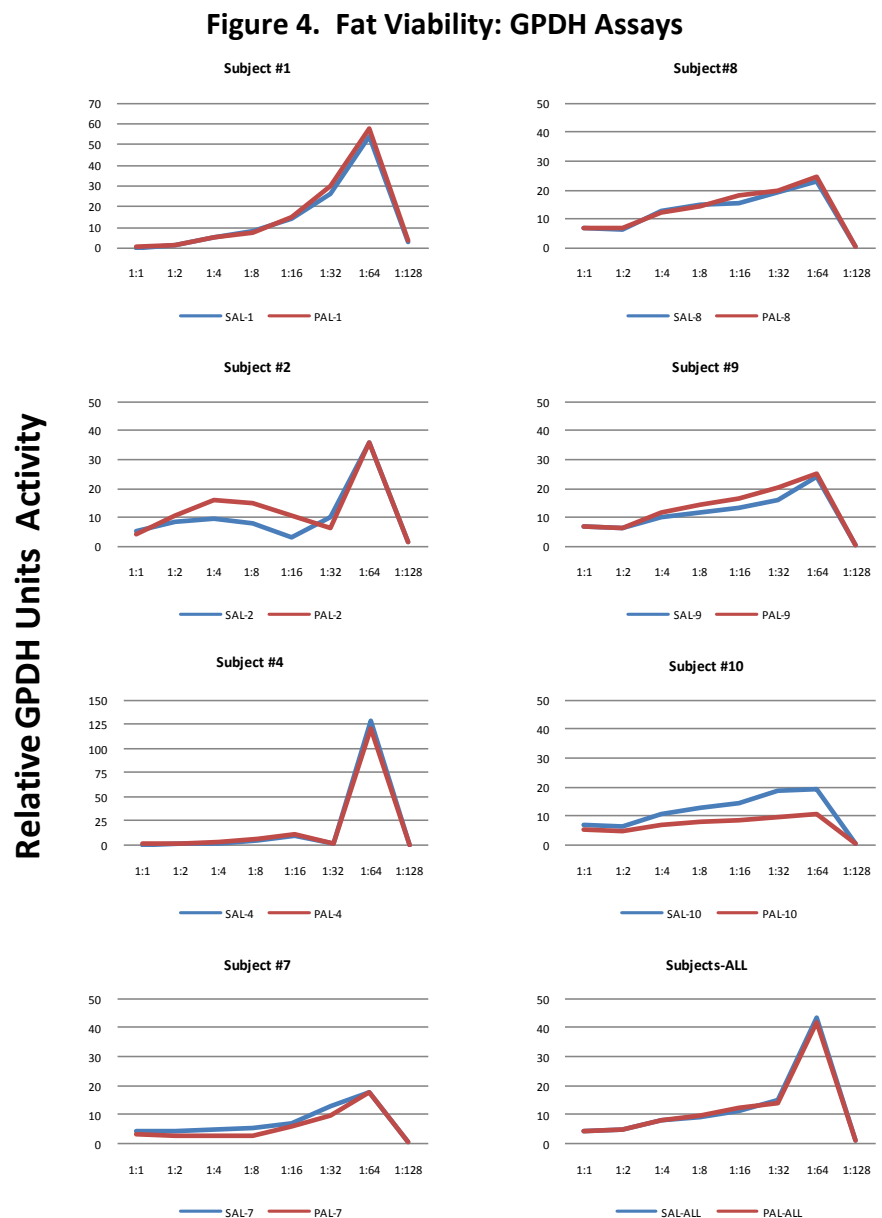
Subject-to-Subject differences in GPDH activity units were evident among the individuals with a range of about 10 to 129 units of activity at the peak. However, SAL and PAL outcomes overlapped in the composite graph and in the majority of individual graphs shown in Figure 4.

The individual and composite results indicated that there were unique Study Subject differences when compared to each other for relative enzyme activity, but no remarkable GPDH activity differences were seen due to the SAL or PAL liposuction harvest method in an individual. That is, the SAL and PAL GPDH composite graphs were essentially super-imposable.

4. ATP Luminescence Assays

The last 3 fat tissue harvests were tested in ATP luminescence tests. The fat was viable in this assay (detailed data not shown) and there was no discernible difference seen between the SAL and PAL groups.

Although the assay worked, ATP luminescence with firefly luciferase readout took a longer time to develop for use, and was a more tedious, longer and expensive assay the GPDH, so all samples were not tested and detailed analyses were not done.

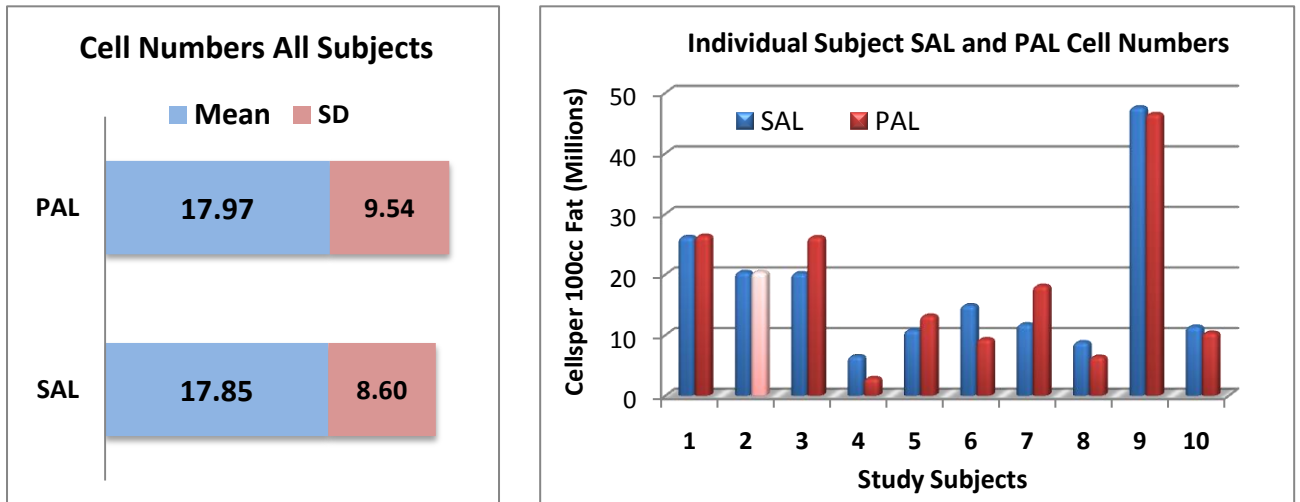


Legend: Enzyme activity for GPDH was tested on harvested fat tissue after two-fold serial dilutions of the fat. Peak activity optimized at 1:64 suggests the presence of inhibitors in the fat. Also, the curves demonstrate clear differences in donor sources but consistency within that donor for 2 harvest sites of the same type of fat.

5. Cell Viability and Numbers

ADSCs from harvested PAL and SAL tissues were assessed for cell numbers post-dissociation from the tissues and in viability tests. Cell viability was demonstrated by cell counts and trypan blue dye exclusion by live cells. Outcomes of assays from all 10 patients are shown in Figure 5.

Figure 5. Cell Numbers for ADSCs from SAL or PAL Tissues

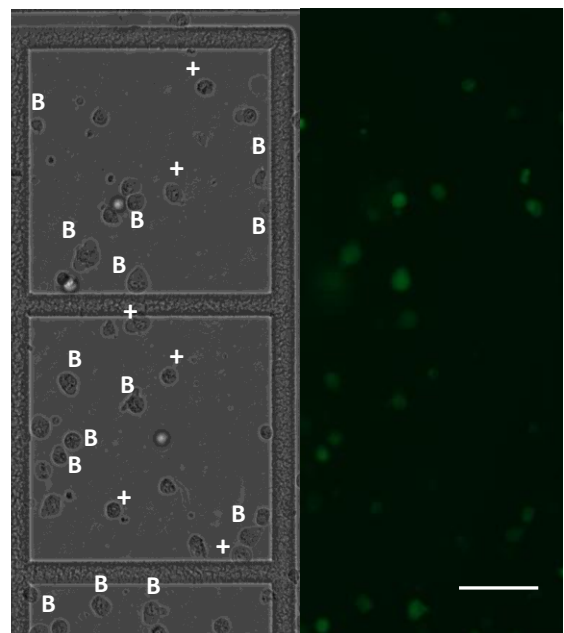


Legend: Total Mean \pm SD cell numbers for 100 cc liposuction fat obtained by SAL (blue bars; 17.85 ± 8.60) or PAL (red bars; 17.70 ± 10.3) were essentially the same (left graph). However, there was variability among the individual patients with regard to cell numbers obtained, but PAL and SAL were generally similar, with neither method outperforming the other. The PAL light red colored bar in Subject 2 shows that the sample was lost in processing.

Isolated ADSCs were either immediately cryopreserved as cell aliquots in EZ-CPZ™ (1:1 ratio in M3D™ medium), then seeded in M3:10™ complete growth medium in culture flasks or multi-well Lab-Teks as monolayer cultures for pre-subculture immunoassays for biomarkers (see section 7), or they were directly tested from the cell suspension.

Subsets of SAL-PAL paired cells were also assayed with Aldefluor®, which is used for the identification, evaluation, and isolation of stem and progenitor cells based on their expression of the enzyme aldehyde dehydrogenase (ALDH), rather than cell surface phenotype. All freshly isolated ADSC populations had comparable fluorescence with about 25-35%(B) Bright staining and 60-70%(+) staining (see example in Figure 6). No demonstrable

Figure 6. Example of Staining of ADSCs with Aldefluor® to Identify Stem Cells



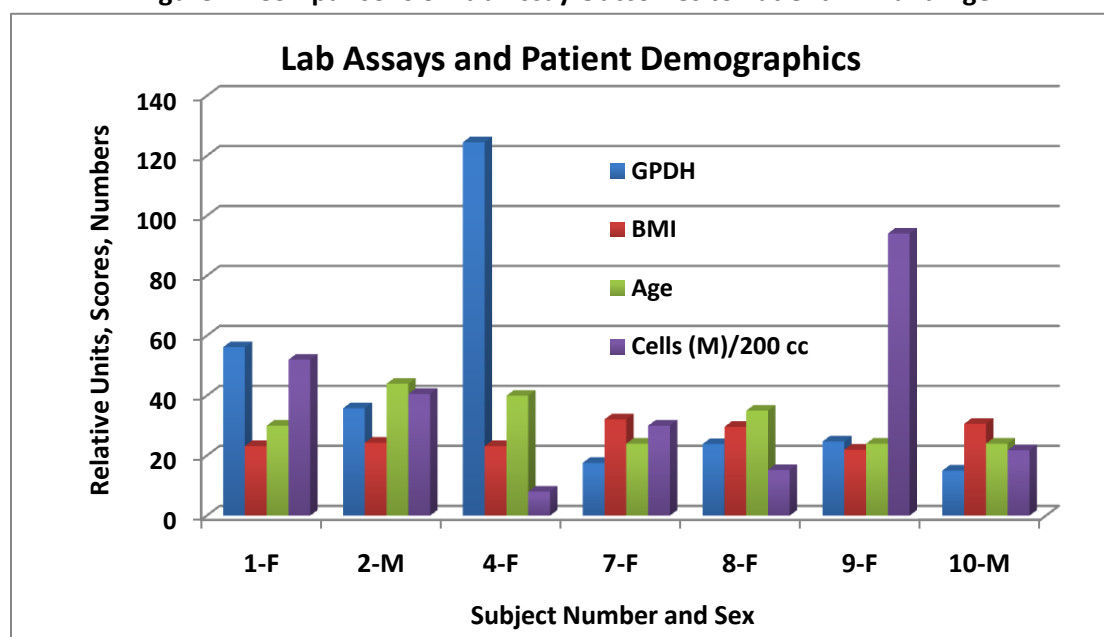
Legend: Light microscopy of cell field (Left Panel). Aldefluor® fluorescence (Right Panel). B=bright staining; +=positive staining. Bar =100 microns.

differences were observed between SAL and PAL ADSC suspensions and the data suggest that 25-70% of ADSCs from an individual donor will be progenitor or stem cells.

6. Correlations of Patient Demographics with Cell Numbers

Laboratory assays for GPDH and cell numbers were compared to sex (indicated on the x axis), BMI and age, as well as other features such as weight (data not shown). As exemplified in Figure 7 for this small sample number of patients there were no correlations among any of these factors, and there were wide ranges of variability.

Figure 7. Comparisons of Lab Assay Outcomes to Patient BMI and Age



7. Cell Expansion and In Vitro Culture

Cells from all of the isolated ADSC samples grew in culture and the SAL and PAL derived cultures were comparable by all the measured parameters from microscopic appearance to the expression of biomarkers (e.g., Figure 8).

ADSC cultures growing in flasks were subcultured no more than 1 to 2 times (usually one to 3 weeks in culture). The cells were then cryopreserved in EZ-CPZ™ (INCELL) in cryovials, then stored in the vapor phase of liquid nitrogen.

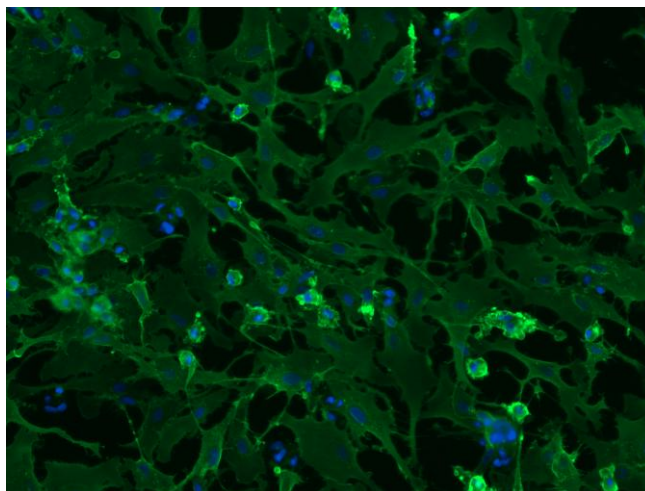
In follow-on work it was demonstrated that all cryopreserved ADSCs re-animated from cryostorage maintained high viability >80%-90% of the viable cells cryopreserved and the ability to re-grow when seeded into culture. Furthermore, they had the ability to regenerate cell populations with growth and multi-lineage differentiation capabilities.

8. Cell Biomarkers

ADSCs from harvested PAL and SAL tissues expressed the tested cell biomarkers CD44, CD105, Stro-1, and CD90 for ADSC and MSC populations in immunocytochemistry assays with specific antibodies. However, except for a single positive cell seen in 2 of the dozens of replicate samples tested, the SAL and PAL ADSCs were consistent in all being negative for the white blood cell marker CD45 (data not shown).

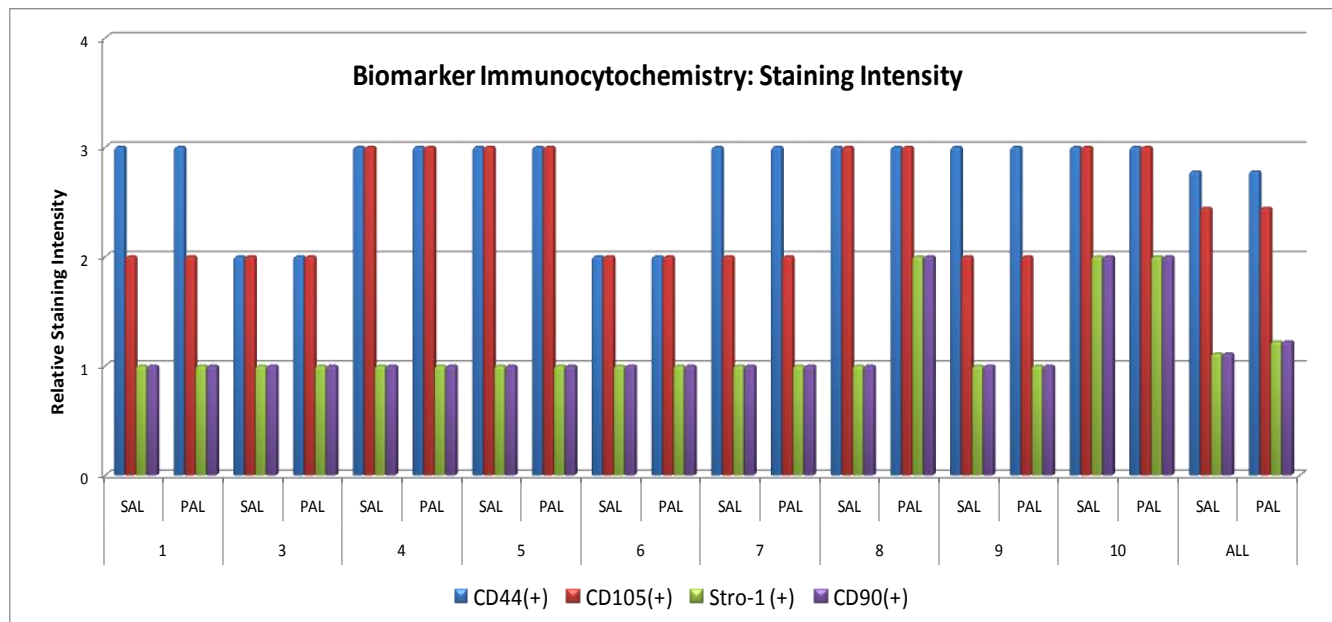
Figure 8 shows the appearance of a CD44 assay of ADSCs designated as “+3” in an observational scoring system of 0 (no staining) to +4 intense staining. Data from each subject plus summary data for all subjects are shown in detail in Figure 9. Note that the cells grow in a typical attached mesenchymal cell pattern and that there are numerous cell surface rounded cells, many in the process of dividing. All SAL and PAL ADSCs had similar in vitro growth potential and general appearance of the cultured cells.

Figure 8. Example of Immunofluorescence Staining of ADSCs for CD44 Biomarker Expression



Legend: ADSCs were seeded onto Lab-Tek slides, allowed to grow for 4 days without subculture, rinsed and stained with FITC-conjugated anti-CD44 antibody (Green cytoplasm) and DAPI (Blue nuclear staining), then visualized with an EVOS fluorescence microscopy. (Magnification, 200X)

Figure 9. Comparative Biomarker Immunoassays



Legend: ADSCs were seeded onto Lab-Tek slides, allowed to grow for 3-5 days without subculture, rinsed and stained with the indicated antibodies either as direct stains or by indirect immunofluorescence with secondary antibodies. Cells were then visualized using an EVOS fluorescence microscope with setting appropriate for the fluorescent dye. Each culture was scored for relative intensity of fluorescence, and the data were tabulated then graphed.

V. DISCUSSION AND CONCLUSIONS

This study compared the viability, quality and quantity of ADSCs derived from fat tissue obtained using PAL or SAL methods on the same day from the same patient. Multiple parameters were analyzed, leading to the conclusion that **there were no significant differences between SAL and PAL methods for adipose tissue or the ADSCs** from that tissue with regard to the following:

- **Tissue Viability** as measured by GPDH enzyme activity or explant cultures;
- **Cell Viability and Numbers** as measured by counts after processing, dye exclusion or Aldefluor® assays;
- **Growth Potential** as measured by in vitro culture, population expansion and the post-cryopreservation re-animation and culture of ADSCs;
- **Biomarker expression** as determined by immunocytochemistry and immunofluorescence for a set of MSC⁵/ADSC-associated biomarkers.

Demonstrable differences and Subject-to-Subject variability were seen with GPDH values and the numbers of ADSCs isolated from tissue normalized to the same volume BETWEEN the Human Subjects but not between the PAL and SAL samples from the same Subject.

Although it was recognized that the total sample size in this study was small, there was no correlation of patients' demographics (age, sex, BMI, weight, etc.) with viability, cell numbers, GPDH or biomarkers. Furthermore, ADSCs from all adipose tissues could be routinely cultured in vitro, expanded and cryopreserved.

An additional goal of the project was to compare surgeon satisfaction in using the SAL and PAL methods. The overall averages look about the same in comparing PAL and SAL (Mean 2.9 +/- 0.72 where a score of 3.0 was "PAL was about the same as SAL"). However, with a breakout of the individual surgeons in these preference questions then the post-surgery interviews revealed that 2 of the 3 surgeons overall preferred the PAL method, whereas the other surgeon preferred the SAL method. No follow-up questions were pursued to help explain these differences beyond the specific questions, but there were a couple of written comments^{6,7} that may be worth following up in future study designs for patient satisfaction.

Finally, the investigators intend to compile this medical and scientific information into a form or forms that can potentially be developed for presentations and for publications in reputable medical or scientific journals.

⁵ MSC: Mesenchymal Stem Cell

⁶ Patient indicated that PAL side more comfortable.

⁷ PAL more comfortable than SAL in the lower abdomen; PAL less comfortable than the SAL in the upper abdomen.